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CA19-9 as a Predictor of Recurrence in Patients With Colorectal Cancer

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Background and Objectives: CA19-9 is a cancer-associated carbohydrate antigen that plays a role in the process of tumor progression as an adhesion molecule.
Methods: We evaluated the prognostic value of CA19-9 tumor expression and CA19-9 preoperative and postoperative serum levels in colorectal cancer patients treated by complete resection. The most powerful discrimination was achieved using the three CA19-9 markers in combination.
Results: CA19-9 tumor expression was identified by immunostaining in 71.0% (86/121) of primary carcinomas. Positive CA19-9 serum levels (⩾37 U/ml) were restricted to cases with positive tumor expression, and CA19-9 was detected more frequently in preoperative serum (20.6%, 25/121) than in 1-month postoperative serum (6.6%, 8/121). Positive tumor expression, positive preoperative serum level, and positive postoperative serum level were all predictive of increased cancer mortality. Patients with three negative parameters had no recurrences and 97.1% 5-year survival, whereas patients with three positive parameters had 62.5% recurrence and 42.8% 5-year survival.
Conclusions: CA19-9 detection in tumor tissue and serum identified patients at high risk of cancer recurrence and death and may be useful in selecting patients for adjuvant therapy.
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KEY WORDS: carbohydrate antigen; metastasis; lymphatic invasion

INTRODUCTION

CA19-9 is a carbohydrate antigen that functions in cancer invasion and metastasis. CA19-9 binds to the endothelial cell-surface receptors E-selectin and P-selectin [1]. Binding to the endothelium is a necessary step in the process of invasion and metastasis. CA19-9 was first detected using a monoclonal antibody raised by immunizing a mouse with a colonic cancer cell line [2]. Circulating CA19-9 antigen has been detected in a variety of cancer patients [3,4]. CA19-9 tumor expression by immunohistochemistry provides prognostic information in colorectal cancer [5], and CA19-9 serum levels may also be helpful in determining prognosis. On the other hand, benign diseases, such as cholecystolithiasis, cholangitis, hepatitis, pancreatitis, and liver cirrhosis are sometimes associated with high CA19-9 levels serum [6].

We have evaluated preoperative and postoperative CA19-9 serum levels and CA19-9 tumor expression in patients with colorectal cancer treated by potentially curative surgery. We wished to assess the prognostic value of three CA19-9 parameters analyzed individually or in combination.

MATERIALS AND METHODS

Patients

During the period 1986–1988, 121 patients with advanced colorectal cancer entered this study. They underwent potentially curative resection in our department in accordance with the General Rules [7]. There were 80 men and 41 women. The mean ages were 59.9 ± 11.4

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(range, 23–85) years. They were staged according to Dukes' classification as modified by Astler-Coller [8]; there were 30 patients in stage B_1, 40 in B_2, 7 in C_1, and 44 in C_2.

Follow-up

All patients underwent standardized follow-up examinations, including laboratory tests every 2 months. Chest roentgenograms, computerized tomography, ultrasound scanning, and barium enemas were performed annually. The mean follow-up duration was 56.0 ± 17.7 (median, 40.7; range, 2–121) months. Recurrence developed in 21.4% (26/121) patients, local recurrence in 9, liver recurrence in 15, and lung recurrence in 11.

Radioimmunoassay

We measured serum CA19-9 levels using a commercially available assay kit (Boehringer Mannheim, Tokyo, Japan) with a recommended cut-off value of 37 U/ml. Serum samples were collected on the day before and about 1 month after surgery.

Immunohistochemistry

We purchased and used NS19-9 monoclonal antibody (TORAY-FUJI Bionics, Tokyo, Japan), which reacts with CA19-9. This antibody was obtained from hybridoma culture supernatants and was diluted with 2% normal swine serum in phosphate-buffered saline. We performed the immunohistochemical study of formalin-fixed, paraffin-embedded blocks of cancer tissue using the avidin-biotin peroxidase method [9]. Two authors judged the result of CA19-9 staining independently and assigned ratings as follows: (−), no expression; (+), expression by less than 50%; and (++), expression by more than 50% of the cells viewed under the microscope.

Statistical Analysis

The results were evaluated using the Chi-squared test, Fisher's exact test, Kaplan Meier method, or generalized Wilcoxon test, as appropriate. Regression coefficients and *P* values were subjected to multivariate analysis using Cox's proportional hazards model. Significance was set at *P* values of less than 0.05.

RESULTS

Presence of CA19-9 Parameters

CA19-9 tumor expression (Ex-CA19-9) was observed in 71.0% (86/121). Elevated preoperative CA19-9 serum levels (Pre-CA19-9) and postoperative CA19-9 serum levels (Post-CA19-9) were detected in 20.6% (25/121) and 6.6% (8/121), respectively. The mean Pre-CA19-9 and Post-CA19-9 levels were 45.5 ± 137.4 U/ml (0.1–1350) and 20.9 ± 52.3 U/ml (1.0–460), respectively.

TABLE I. Relationship Among CA19-9 Parameters in Patients With Colorectal Cancer*

	Pre-CA19		Post-CA19-9	
	Mean ± SD	Positive/ total[a]	Mean ± SD	Positive/ total[a]
Ex-CA19-9				
(−)	7.6 ± 7.9	0/35 (0)	7.6 ± 7.6	0/35 (0)
(+)	20.6 ± 24.1	7/49 (14.2)	19.1 ± 41.6	3/49 (6.1)
(++)	114.4 ± 234.4	18/37 (48.6)	35.9 ± 79.8	5/37 (13.5)

*Cut-off: CA19-9 ⩾ 37 U/ml.
[a]Percent in parentheses.

Relationship of CA19-9 Parameters

Pre-CA19-9 ($P < 0.01$) and Post-CA19-9 ($P < 0.01$) levels correlated significantly with Ex-CA19-9 levels (Table I). All Ex-CA19-9 (−) patients were both pre-CA19-9 (−) and Post CA19-9 (−). All Pre-CA19-9 (−) patients were Post-CA19-9 (−). All Post-CA19-9 (+) patients were both Ex-CA19-9 (+ or ++) and Pre CA19-9 (+) (Fig. 1).

CA19-9 Parameters and Clinicopathologic Factors

We investigated the relationship between CA19-9 parameters and clinicopathologic factors: patient age, sex, carcinoembryonic antigen (CEA) levels, tumor size, tumor site, macroscopic type, circumference, histologic type, depth of invasion, lymph node metastasis, lymphatic invasion, venous invasion, and pattern of growth.

Ex-CA19-9 correlated with lymph node metastasis ($P < 0.01$), whereas Pre-CA19-9 was determined by tumor size ($P < 0.01$), depth of invasion ($P < 0.01$), lymph node metastasis ($P < 0.01$), lymphatic invasion ($P < 0.05$), and pattern of growth ($P < 0.01$).

Elevation in serum levels of CEA (Pre-CEA) was detected preoperatively in 39.5%. The mean Pre-CEA was 8.9 ± 19.1 (0.2–240) U/ml. There was no correlation between preoperative CA19-9 and CEA serum levels ($r = 0.27$, NS), probably because their chemical structures and biological expression are different.

CA19-9 Parameters and Recurrence

Pre-CA19-9 ($P = 0.0001$), Post-CA19-9 ($P = 0.013$), and Ex CA19-9 ($P = 0.00061$) levels correlated with recurrence. Ex-CA19-9 (−) patients had no recurrences. On the other hand, Ex-CA19-9 (+ and ++) patients had increased recurrence in line with the extent of serum CA19-9 levels (Table II). These three CA19-9 parameters also correlated significantly with hematogenous recurrence, such as liver and lung recurrence, respectively.

CA19-9 Parameters and Survival by Univariate Analysis

Pre-CA19-9 ($P = 0.000095$), Post-CA19-9 ($P = 0.0069$), and Ex-CA19-9 ($P = 0.0058$) levels correlated

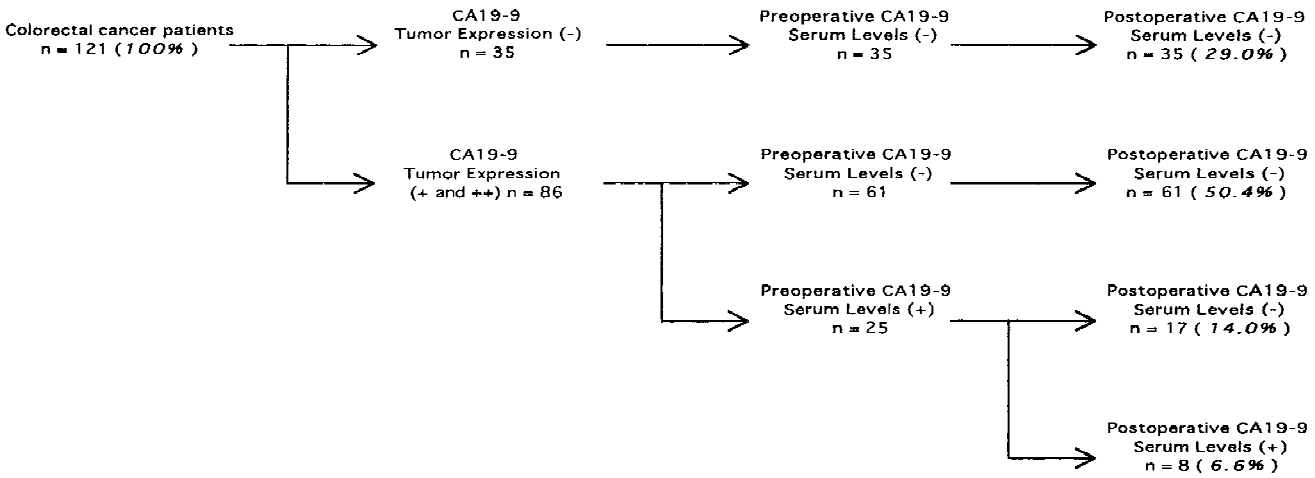

Fig. 1. Relationship of all CA19-9 parameters. All Ex-CA19-9 (−) patients were both Pre-CA19-9 (−) and Post-CA19-9 (−). All Pre-CA19-9 (−) patients were Post-CA19-9 (−). All Post-CA19-9 (+) patients were both Ex-CA19-9 (+ and ++) and Pre CA19-9 (+).

TABLE II. Colorectal Cancer: Recurrence in Relation to CA19-9 Parameters*

	Pre-CA19-9(−)/ Post-CA19-9(−)	Pre-CA19-9(+)/ Post-CA19-9(−)	Pre-CA19-9(+)/ Post-CA19-9(+)
Ex-CA19-9			
(−)	0 (0/35)	(0/0)	(0/0)
(+)	16.6 (7/42)	25.0 (1/4)	33.3 (1/3)
(++)	31.5 (6/19)	53.8 (7/13)	80.0 (4/5)

*Percents (positive/total) are given in parentheses.

TABLE III. Colorectal Cancer: CA19-9 Parameters and 5-Year Survival Rate

	5-Year survival according to CA19-9 status[a]		
	(−)	(+)	*P* value[b]
CA19-9 expression	97.1	76.4	0.0058
Preoperative CA19-9 levels	88.4	58.3	0.00095
Postoperative CA19-9 levels	84.8	42.8	0.0069

[a]Data are percents.
[b]By the Wilcoxon test.

with survival (Table III). Increase in CA19-9 parameters related to Astler-Coller stage, but these differences did not reach statistical significance.

Ex-CA19-9 (−) patients had no recurrences and a good prognosis (97.1% of the 5-year survivors). Even for Ex-CA19-9 (+ and ++) patients, negative CA19-9 serum levels indicated a better prognosis than Pre-CA19-9 (+) ($P = 0.022$) and Post-CA19-9 (+) ($P = 0.041$) (Fig. 2).

Pre-CA19-9 (+) patients had a worse survival than Ex-CA19-9 (+) patients. Even for Pre-CA19-9 (+) patients, Post-CA19-9 (−) indicated a better prognosis than Post-CA19-9 (+). Post-CA19-9 (+) patients had the worst survival (Table IV).

CA19-9 Parameters and Survival by Multivariate Analysis

Multivariate analysis was performed for patient age, sex, CEA levels, tumor size, macroscopic type, histologic type, depth of invasion, lymph node metastasis, lymphatic invasion, venous invasion, pattern of growth, and CA19-9 parameters.

Multivariate analysis of survival correlated significantly with depth of invasion and lymph node metastasis, although CA19-9 parameters showed no such correlation.

Cases of Positive Postoperative CA19-9 Serum Levels

Of the eight post-CA19-9 (+) patients, five had colon and three had rectum disease (Table V). They were all stage T-3 and had five lymph node metastases. Positive CEA serum levels (≥5 U/ml) were detected in 50% (4/8). They were both Ex-CA19-9 (+ or ++) and Pre-CA19-9 (+). Recurrence developed in 62.5% (5/8) within 18 months. All patients with recurrent disease had hematogenous metastasis. Patients without recurrence have received strict follow-up.

DISCUSSION

Attachment between cancer cells and endothelial cells is an important process in the development of tumor metastasis. CA19-9 is a well recognized and utilized tumor marker for colorectal cancer [3]. CA19-9 is a type of sialylated lacto-N-fucopentose II expressed on the cell surface of many colorectal cancer cells. CA19-9 has been demonstrated to serve as a ligand for endothelial leukocyte adhesion molecule-1 (ELAM-1). Cells expressing CA19-9 adhere to endothelial cells activated by cyto-

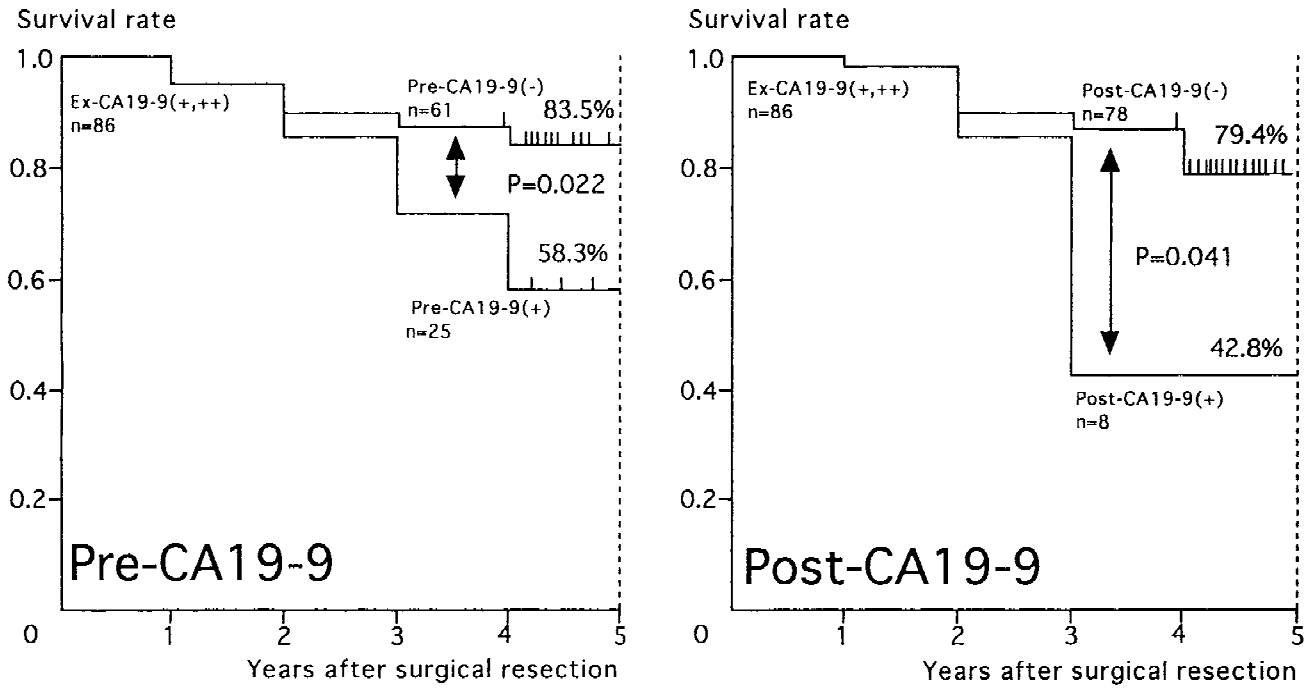

Fig. 2. Survival curves in positive tumor CA19-9 expression patients (n = 86). Positive serum CA19-9 patients had a poorer prognosis than negative serum CA19-9 patients preoperatively and postoperatively.

TABLE IV. Colorectal Cancer: 5-Year Survival Rate in Relation to CA19-9 Parameters*

	Pre-CA19-9(−)/ Post-CA19-9(−)	Pre-CA19-9(+)/ Post-CA19-9(−)	Pre-CA19-9(+)/ Post-CA19-9(+)
Ex-CA19-9			
(−)	97.1 (35)	(0)	(0)
(+)	85.7 (42)	75.0 (4)	50.0 (3)
(++)	78.9 (19)	61.5 (13)	40.0 (5)

*Data are percents, with numbers in parentheses.

kines. CA19-9 may actually play a role in the adhesion of cancer cells to endothelial cells, resulting in hematogenous metastasis [1].

In tissues, CA19-9 is expressed as a monosialoganglioside, whereas in serum CA19-9 is found on mucin-type glycolipids [10]. CA19-9 is drained by the thoracic ducts of the lymphatic system in patients with gastrointestinal cancer [11]. We have demonstrated that CA19-9 parameters correlated significantly with lymph node metastasis and lymphatic invasion. CA19-9 expression was significantly stronger in the metastases to regional lymph nodes than in the primary lesions [5]. However, no one has elucidated fully the process by which CA19-9 expression correlates with lymphatic dissemination.

MUC1 apomucin is a glycoprotein that carries CA19-9 antigen [12]. The levels of glycosylated MUC1 mucins correlates with tumor progression to the advanced stage of colorectal carcinoma [13]. Mucins are the major epithelial luminal surface glycoproteins and are characterized by high molecular masses and high carbohydrate side chain content [14]. The cloning of several genes (MUC1-7) encoding human mucins constitutes a major step toward understanding the structure, heterogeneity, polymorphism, and function of the peptide mucin core [15]. CA19-9 serum levels seem to be more sensitive predictors for recurrence than CA19-9 tumor expression, because patients with positive CA19-9 serum levels had a worse prognosis than patients with positive CA19-9 tumor expression. These results suggest that CA19-9 serum levels, namely CA19-9 mucin-type glycolipids in serum, have a more powerful influence on prognosis than CA19-9 tumor expression.

CA19-9 serum levels were low sensitive for cancer patients, partly because the antigenic determinant of CA19-9 is a sialylated derivative of the Lewis^{a} blood group antigen [10]; genotypically, Lewis$^{a\text{-}b\text{-}}$ individuals (about 5–10% of the general population) cannot synthesize CA19-9 antigen.

CA19-9 parameters failed to correlate with survival according to Astler-Coller stage and multivariate analysis. Multivariate analysis showed that survival was significantly related to depth of invasion and lymph node metastasis. However, we found that the trend toward survival decreased with increasing CA19-9 parameters related to Astler-Coller stage. Because our numbers are small, we need to investigate more cases to assess whether CA19-9 antigens are clearly shed by primary tumor

TABLE V. Cases of Positive Postoperative CA19-9 Serum Levels in Cancer of the Colon and Rectum*

Patient no.	Age (yr)	Sex	Location	Stage		LI	VI	CEA	CA19-9			Recurrence (site)
				T	N				Ex.	Pre.	Post.	
1	23	F	Colon	3	1	+	−	25.9	++	960	220	Lung
2	57	M	Rectum	3	1	+	−	105.5	++	440	54	Liver, local
3	75	F	Rectum	3	1	+	+	9.3	++	67	44	Lung, local
4	71	F	Rectum	3	1	+	+	3.8	++	42	44	Liver, local
5	59	M	Colon	3	0	−	−	3.3	+	88	298	Liver
6	27	M	Colon	3	0	+	−	2.8	++	1,350	460	None
7	67	M	Colon	3	1	−	−	3.5	+	40	42	None
8	52	F	Colon	3	0	+	−	14.9	+	48	43	None

*LI, lymphatic invasion; VI, vessel invasion; Ex., tumor expression; Pre., preoperative serum levels; Post., postoperative serum levels.

and are commonly elevated with increasing stage irrespective of the risk of recurrence.

Many patients develop recurrences within a few years after curative resection [16]. The main cause of recurrence may be the growth of micrometastases already established before resection [17]. Cancer micrometastases may be present in patients whose CA19-9 serum levels remain elevated and then release CA19-9. These data suggest that patients whose preoperative CA19-9 levels changed to negative postoperatively may have only a primary tumor, whereas patients whose levels did not change may have a primary tumor and micrometastases. Recurrence of colorectal cancer results in an extremely poor prognosis. The assessment of CA19-9 may be helpful when deciding on a patient's management, because CA19-9 parameters correlated significantly with recurrence, and a positive CA19-9 phenotype has a strong metastatic potential.

Hematogenous recurrence is the most important factor influencing the survival of colorectal cancer patients [18]. An autopsy study has indicated that 60% of colorectal cancer patients show microscopic evidence of liver metastasis after curative resection [19]. Early detection of recurrence may improve the resectability of recurrent cancers. Early surgical removal of recurrence provides patients with disease-free and long-term survival [20]. Our study revealed that CA19-9 parameters correlated significantly with recurrence, especially hematogenous recurrence. CA19-9 as a ligand for E-selectin may relate to the fact that serum CA19-9 levels were elevated in patients with hematogenous recurrence.

A majority of colorectal cancers expressed CA19-9 tumor staining (71.0%), whereas a minority showed elevated CA19-9 serum levels preoperatively (20.6%) and postoperatively (6.6%). However, risk of cancer recurrence and death increased in accordance with the extent of CA19-9 parameters. A combination of CA19-9 parameters could identify and discriminate high-risk patients in colorectal cancer. To increase the informative value, we should evaluate CA19-9 tumor expression and measure CA19-9 serum levels preoperatively and postoperatively. We recommend that colorectal cancer patients with Post-CA19-9 (+) and both Pre-CA19-9 (+) and Ex-CA19-9 (++) receive aggressive adjuvant chemotherapy with strict follow-up surveillance.

We conclude that CA19-9 detection in tumor tissue and serum identifies patients at high risk of cancer recurrence and death and may be useful in selecting patients for adjuvant therapy.

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